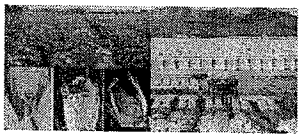
APPENDIX 2

United States Department Of Agriculture
Agricultural Research Service



Title: Somatic Embryo Initiation and Germination in Diploid Cotton (Gossypium Arboreum L.)

Authors

- Sakhanokho, Hamidou - UNIVERSITY OF GEORGIA
- Zipf, Allan - ALABAMA A&M UNIVERSITY
- Rajasekaran, Kanniah - *rajah*
- Saha, Sukumar
- Sharma, Govind - ALABAMA A&M UNIVERSITY
- Chee, Peng - UNIVERSITY OF GEORGIA

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Interpretive Summary: Rapid progress is being made in techniques of plant genetic engineering. Suitable plant regeneration method is an essential prerequisite for successfully accomplishing the goals of genetic engineering. The diploid cotton species are not only the reservoir of important pest and disease resistant genes, and improved agronomic and fiber traits but also offer better opportunities to study gene structure and function through advance techniques of gene knockouts. To our knowledge, there is no report on somatic embryogenesis and plant regeneration from the diploid cultivated species of cotton. Here we report a suitable method to produce considerable amounts of somatic embryos and plant regeneration in Gossypium arboreum L., one of the cultivated diploid cotton species. Carbohydrate source and concentration levels were evaluated to improve somatic embryo (SE) production and desiccation treatments to improve the conversion efficiency of SEs to plants in a diploid G. arboreum accession, A2-9 (PI-529712). Large numbers of SEs induced or their conversion to plantlets were achieved with an MS/sucrose-based medium M2 [0.04 M sucrose, 0.3 uM NAA] that produced 219 embryos g-1, and close to 11% of these embryos germinated into plantlets. In a second experiment, when immature G. arboreum SEs indiced on M1 [0.2 M glucose, 2.6 uM NAA, and 0.2 uM kinetin] medium underwent a 3-day desiccation treatment, 49% of these immature SEs were converted to plantlets after a 4-week period on M2 medium. These improved results can help pave the way for the future genetic transformation and associated gene structure and function studies utilizing G. arboreum.

Technical Abstract: The diploid cotton species can constitute not only a valuable gene pool for the more agronomically desirable cultivated tetraploid cultivars but also offer better opportunities to study gene structure and function through gene knockouts. In order to exploit these advantages, a regeneration system is required to achieve these transformation-based goals. Carbohydrate source and concentration levels were evaluated to improve somatic embryo (SE) production and desiccation treatments to improve the conversion efficiency of SEs to plants in a diploid G. arboreum accession, A2-9 (PI-529712). Neither a 5-day desiccation treatment of embryogenic callus previously cultured in liquid medium nor

filter paper insertion improved the numbers of SEs induced or their conversion to plantlets. However, better results were achieved with a MS/sucrose-based medium M2 [0.04 M sucrose, 0.3 uM NAA] that produced 219 embroys g-1, and close to 11% of these embryos germinated into plantlets. In a second experiment, when immature G. arboreum SEs induced on M1 [0.2 M glucose, 2.6 uM NAA, and 0.2 uM kinetin] medium underwent a 3-day desiccation treatment, 49% of these immature SEs were converted to plantlets after a 4-week period of M2 medium. These improved results can help pave the way for the future genetic transformation and associated gene structure and function studies utilizing G. arboreum. These results, in particular the 3-day desiccation treatment, can also be incorporated into regeneration protocols to help improve the regeneration efficiency of other Gossypium species.

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